510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K113433

B. Purpose for Submission:

To obtain substantial equivalence for Simplexa™ *C. difficile* Universal Direct assay

C. Measurand:

Clostridium difficile toxin B gene (tcdB)

D. Type of Test:

Real time PCR

E. Applicant

Focus Diagnostics Inc.

F. Proprietary and Established Name:

Simplexa™ C. difficile Universal Direct Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OMN	I	21 CFR 866.2660	83 - Microbiology

H. Intended Use:

1. Intended use:
The Focus Diagnostics Simplexa™ *C. difficile* Universal Direct is a real-time polymerase chain reaction (PCR) assay and is intended for use on the 3M Integrated Cycler instrument for the detection of toxigenic *Clostridium difficile* toxin B gene (*tcdB*) in liquid or unformed stool samples from individuals suspected of *C. difficile* infection. This test aids in the diagnosis of *Clostridium difficile* associated disease (CDAD).

2. Indications for use:
The Focus Diagnostics Simplexa™ *C. difficile* Universal Direct is a real-time polymerase chain reaction (PCR) assay and is intended for use on the 3M Integrated Cycler instrument for the detection of toxigenic *Clostridium difficile* toxin B gene (*tcdB*) in liquid or unformed stool samples from individuals suspected of *C. difficile* infection. This test aids in the diagnosis of *Clostridium difficile* associated disease (CDAD).

3. Special conditions for use statement:

   For Prescription Use

4. Special instrument requirements:

   3M Integrated Cycler Instrument

I. Device Description:

The test is a real-time polymerase chain reaction (PCR) amplification and detection system that utilizes bi-functional fluorescent probe-primers for the detection of *C. difficile* in liquid or unformed stool. The Focus Diagnostics Simplexa™ *C. difficile* Universal Direct assay contains sufficient reagents for 100 reactions. Kit components are listed below.

<table>
<tr><th>Kit Component</th><th>Component Description</th></tr>
<tr><td>Simplexa™ C. difficile Primer Mix</td><td>Bi-functional fluorescent probe-primers specific for detection of C. difficile and for the DNA Internal Control including DNA Internal Control template
<table>
<tr><th>Target</th><th>Probe Fluorophore</th><th>Excitation (nm)</th><th>Emission (nm)</th><th>Targeted Gene</th></tr>
<tr><td>C. difficile</td><td>FAM</td><td>495</td><td>520</td><td>Toxin B</td></tr>
<tr><td>IC</td><td>Q670</td><td>644</td><td>670</td><td>N/A</td></tr>
</table></td></tr>
<tr><td>Simplexa™ Master Mix</td><td>DNA polymerase, buffer and dNTPs</td></tr>
<tr><td>Simplexa™ C. difficile Positive Control (PC)</td><td>Clostridium difficile Genomic DNA</td></tr>
<tr><td>Simplexa™ C. difficile Barcode Card</td><td>Assay specific parameters</td></tr>
</table>

J. Substantial Equivalence Information:

1. Predicate device names:

   a. BD GeneOhm *C.diff.* Assay

   b. **illumi**gene *C. difficile* Assay

   c. Bartel's cytotoxicity assay for *Clostridium*

2. Predicate K numbers:

   a. K081920

b. K110012

c. K833447

3. Comparison with predicate:

Comparison is to BD GeneOhm *C. diff* Assay (K081920)

Similarities		
Item	Device	Predicate
Intended Use	Detection of *C. difficile* toxin B gene	Same
Matrix	Liquid or uniformed stool samples	Same
Technology	Assay based on real time PCR	Same

Differences		
Item	Device	Predicate
Instrumentation	Test is performed on the 3M Integrated Cycler	Test uses the Cepheid Smart Cycler
Detection techniques	PCR with bifunctional fluorescent primer-probes	PCR with molecular beacons
Reference Method	Toxigenic Culture	Cytotoxicity Assay

K. Standard/Guidance Document Referenced:

Draft Guidance for Industry and Food and Drug Administration Staff - Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of *Clostridium difficile*. November 2010.

L. Test Principle:

The test is a real-time polymerase chain reaction (PCR) amplification and detection system that utilizes bi-functional fluorescent probe-primers for the detection of *C. difficile* in liquid or unformed stool. The assay is composed of two principal steps: (1) Heat treatment of stool samples, (2) Amplification of the *C. difficile* DNA and internal control DNA using bi-functional fluorescent probe-primers together with reverse primers. The DNA internal control is used to monitor potential presence of PCR inhibitors. The assay targets a sequence which is in a well conserved region of *C. difficile* toxin B gene (*tcdB*).

The 3M Integrated Cycler is a rapid real-time Polymerase Chain Reaction thermocycler used for the identification of nucleic acid from prepared biological samples. The instrument utilizes disc media to contain and process samples. The instrument uses real time flourometric detection to identify targets within the sample wells. The instrument is controlled by an external computer running the Integrated Cycler Studio Software.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Inter-laboratory reproducibility and inter/intra-assay reproducibility were assessed at three sites. Each of the three sites used the same panel, which consisted of contrived samples in stool-TE buffer matrix spiked with *C. difficile* bacterial stock. The panel included a high negative, low positive and medium positive sample. Each site utilized at least two testing operators and one lot of Simplexa™ *C.difficile* Universal Direct kit across five days. On each day two runs were performed, one by each operator. These results met the acceptance criteria and are acceptable. A summary of the reproducibility results are shown in the table below.

Sample	Site 1			Site 2			Site 3			Total Agreement with Expected Results	95% CI
	Agreement with Expected Results	Avg. Ct	Total %CV	Agreement with Expected Results	Avg. Ct	Total %CV	Agreement with Expected Results	Avg. Ct	Total %CV		
Low Positive	30/30	35.20	1.64	30/30	35.26	1.30	30/30	35.30	2.04	100% (90/90)	95.9% - 100.0%
[1]Medium Positive	29/29	32.71	0.82	30/30	32.60	1.07	30/30	32.65	0.77	100% (89/89)	95.9% - 100.0%
[2]Positive Control (PC)	30/30	32.55	1.11	29/29	32.13	0.87	31/31	32.33	0.63	100% (90/90)	95.9% - 100.0%
High Negative	29/30			30/30			30/30			98.9% (89/90)	94.0% - 99.8%
[3]No Template Control (NTC)	30/30			30/30			29/30			98.9% (89/90)	94.0% - 99.8%
Total Agreement	148/149 (99.3%)			149/149 (100.0%)			150/151 (99.3%)			447/449 (99.6%)	98.4% - 99.9%

[1]One replicate was declared "invalid" based on the site operator discretion. It was "Not Detected".
[2]One replicate was "Invalid" at Site 2 and additional replicate was tested in Run-1, Day-1 at Site 3 because the site had thought that one of the three replicates had a 'bubble' and therefore as a precaution loaded an additional replicate at the end of one of the run.
[3]One replicate of the NTC is "Detected" and may be attributed to possible contamination due to handling.
Note: Two samples – "NTC" and "High Negative" were excluded from reporting Quantitative Reproducibility Results.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Quality control ranges have been established as indicated in the table below. If the controls are not within these parameters, patient results should be considered invalid and the assay repeated. Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

Expected Control Ranges

Control Type	Simplexa™ *C. difficile* Ct value
No Template Control (NTC)	Ct = 0
Positive Control (PC)	Ct ≤40, ≠0

d. Detection limit:

The Limit of Detection (LoD) was determined for the Simplexa™ *C. difficile* Universal Direct assay by performing limiting dilution studies using bacterial stocks of two strains of each *C. difficile* bacteria strain. The strains (ATCC 43255 and NAP 1A) were cultured and quantified. The LoD was determined using one lot of the Simplexa™ *C. difficile* Universal Direct Kit. Tentative LoD was determined using three replicates in screening followed by confirmation using twenty replicates. LoD was determined to be 560.7 CFU/mL or 1.12 CFU/PCR for strain ATCC 43255 and 76.3 CFU/mL or 0.15 CFU/PCR for strain NAP 1A. These results met the acceptance criteria and are acceptable.

e. Analytical specificity:

Cross-Reactivity

Analytical specificity for various possible cross-reactants was performed. A total of 119 potential cross-reactants were tested. No cross-reactivity was observed as shown in the Table below.

Tested Cross-Reactants			
No.	**Cross Reactant**	**Concentration**	**Result**
1	*Abiotrophia defective*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
2	*Acinetobacter baumanii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
3	*Acinetobacter lwofii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
4	Adenovirus 40	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
5	*Aeromonas hydrophila*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
6	*Alcaligenes faecalis* subsp. *Faecalis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
7	*Anaerococcus tetradius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
8	*Bacillus cereus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
9	*Bacteroides caccae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
10	*Bacteroides merdae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
11	*Bacteroides stercoris*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
12	*Bifidobacterium adolescentis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
13	*Bifidobacterium longum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
14	*Campylobacter coli*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
15	*Campylobacter jejuni*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
16	*Candida albicans*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
17	*Candida catenulate*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
18	*Cedecea davisae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
19	*Chlamydia trachomatis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
20	*Citrobacter amalonaticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	**Cross Reactant**	**Concentration**	**Result**
21	*Citrobacter freundii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
22	*Citrobacter koseri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
23	*Citrobacter sedlakii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
24	*Clostridium beijerinckii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
25	*Clostridium bifermentans*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
26	*Clostridium bolteae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
27	*Clostridium butyricum*	6.80×10^5 cfu/mL	No Cross Reactivity Observed
28	*Clostridium chauvoei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
29	*Clostridium difficile* non-toxigenic ATCC 43593	1.00×10^6 cfu/mL	No Cross Reactivity Observed
30	*Clostridium difficile* non-toxigenic ATCC43601	1.00×10^6 cfu/mL	No Cross Reactivity Observed
31	*Clostridium fallax*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
32	*Clostridium histolyticum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
33	*Clostridium innocuum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
34	*Clostridium methylpentosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
35	*Clostridium nexile*	6.90×10^5 cfu/mL	No Cross Reactivity Observed
36	*Clostridium novyi*	8.90×10^5 cfu/mL	No Cross Reactivity Observed
37	*Clostridium paraputrificum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
38	*Clostridium perfringens*	6.70×10^5 cfu/mL	No Cross Reactivity Observed
39	*Clostridium ramosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
40	*Clostridium scindens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
41	*Clostridium sepiticum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
42	*Clostridium sordellii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
43	*Clostridium sphenoides*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
44	*Clostridium sporogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
45	*Clostridium symbiosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
46	*Clostridium terdium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
47	*Clostridium tetani*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
48	*Collinsella aerofaciens*	8.60×10^5 cfu/mL	No Cross Reactivity Observed
49	*Corynebacterium genitalium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
50	Coxsackie virus A16	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
51	Cytomegalovirus AD-169	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
52	*Desulfovibrio piger*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
53	Echovirus 9	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
54	*Edwardsiella tarda*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
55	*Eggerthellalenta*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
56	*Enterobacter aerogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
57	*Enterobacter cloacae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
58	*Enterococcus raffinosus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
59	*Enterococcus casseliflavus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
60	*Enterococcus cecorum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
61	*Enterococcus dispar*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
62	*Enterococcus hirae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
63	*Enterococcus faecalis* vanB	1.00×10^6 cfu/mL	No Cross Reactivity Observed
64	*Enterococcus faecium* vanA	1.00×10^6 cfu/mL	No Cross Reactivity Observed
65	*Enterococcus gallinarum* vanC	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	**Cross Reactant**	**Concentration**	**Result**
66	Enterovirus 71	5.01×10^4 $TCID_{50}$/mL	No Cross Reactivity Observed
67	*Escherichia coli*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
68	*Escherichia fergusonii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
69	*Escherichia hermannii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
70	*Fusobacterium varium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
71	*Gardnerella vaginalis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
72	*Gemella morbillorum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
73	*Hafnia alvei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
74	*Helicobacter pylori*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
75	*Homo sapiens*	3.07 pg/mL	No Cross Reactivity Observed
76	*Klebsiella oxytoca*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
77	*Klebsiella pneumoniae* subsp. *Pneumoniae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
78	*Lactobacillus acidophilus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
79	*Lactobacillus reuteri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
80	*Lactococcus lactis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
81	*Leminorela grimontii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
82	*Listeria grayi*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
83	*Listeria innocua*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
84	*Listeria monocytogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
85	Norovirus Group I (recombinant)	8.13×10^4 $TCID_{50}$/mL	No Cross Reactivity Observed
86	*Peptoniphilus asaccharolyticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
87	*Peptostreptococcus anaerobius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
88	*Plesiomonas shigelloides*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
89	*Porphyromaonas asaccharolytica*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
90	*Prevotella melaninogenica*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
91	*Proteus mirabilis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
92	*Proteus penneri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
93	*Providencia alcalifaciens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
94	*Providencia rettgeri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
95	*Providencia stuartli*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
96	*Pseudomonas aeruginosa*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
97	*Pseudomonas putida*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
98	Rotavirus, Strain Wa	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
99	*Salmonella enterica* subsp. *Arizonae* (formerly *Choleraesuis arizonae)*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
100	*Salmonella enterica* subsp. *Choleraesuis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
101	*Salmonella enterica* subsp. *Enterica* serovar *Typhimurium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
102	*Serratia liquefaciens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
103	*Serratia marcescens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
104	*Shigella boydii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
105	*Shigella dysenteriae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
106	*Shigella sonnei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
107	*Staphylococcus aureus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
108	*Staphylococcus epidermidis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
109	*Stenotrophomonas maltophilia*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
110	*Streptococcus agalactiae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	**Cross Reactant**	**Concentration**	**Result**
111	*Streptococcus dysgalactiae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
112	*Streptococcus intermedius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
113	*Streptococcus uberis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
114	*Trabulsiella guamensis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
115	*Veillonella parvula*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
116	*Vibrio cholerae*	4.10×10^{-3} pg/mL	No Cross Reactivity Observed
117	*Vibrio parahaemolyticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
118	*Yersinia bercovieri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
119	*Yersinia rohdei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Note:

1. Total 10 runs were performed to test 119 cross reactants in triplicate. Additionally, each run included five replicates of baseline (un-spiked) sample.
2. Each replicate of all 119 cross-reactants and baseline samples were "Not Detected".

Interference:

The performance of this assay was evaluated with potentially interfering substances that may be present in stool specimens at the concentrations indicated in the Table below. A total of 21 potentially interfering substances were tested. No Interference was observed.

Interferents	**Active Ingredient**	**Interferent Concentration**	**Dete**	
			***C. difficile* Strain - ATCC4325**	***C. difficile* Strain - NAP 1**
1% Hydrocortisone Cream	Hydrocortisone	2% (w/v)	3	3
Alleve	Naproxen	14 mg/ml	3	3
Antacid and Anti-gas generic	Aluminum Hydroxide, Magnesium Hydroxide	0.1 mg/ml	3	3
Antacid Generic	Calcium Carbonate	0.1 mg/ml	3	3
Barium sulfate	Barium sulfate	5 mg/ml	3	3
Fleet	Mineral Oil	2% (v/v)	3	3
Imodium AD	Loperamide	0.005 mg/ml	3	3
KY Jelly	Glycerin	2%(w/v)	3	3
Laxative generic	Sennosides	0.1 mg/ml	5	3
Metronidazole	Metronidazole	14 mg/ml	3	3
Milk of Magnesia	Magnesium Hydroxide	0.2 mg/ml	3	3
Moist towelettes	Benzalkonium Chloride	10%(v/v)	3	3
Mucin	Mucin	3 mg/ml	3	3
Nystatin	Nystatin	10000 USP units/ml	3	3
Palmitic acid	Palmitic acid	2 mg/ml	3	3
Pepto-Bismol	Bismuth Subsalicylate	0.175 mg/ml	3	3
Preparation H	Phenylephrine	2% (w/v)	3	3
Stearic acid	Stearic Acid	4 mg/ml	3	3
Trojan with nonoxynol-	Nonoxynol-9	1.4 mg/ml	3	3
Vancomycin	Vancomycin	1.4 mg/ml	5	3
Whole blood	Whole blood	3%	3	7

*One replicate reported as "Invalid" due to IC failure in initial run of three replicates. All three replicates reported as "Detected" in repeat run.

**One replicate reported as "Not Detected" in initial run of three replicates. However all five replicates reported as "Detected" in repeat run.

Analytical reactivity of additional strains of *C. difficile* was evaluated in negative stool-TE buffer matrix. Quantified bacterial material was spiked into the negative stool-TE buffer matrix at a single dilution. A total of 20 different strains were tested in triplicate. All of the tested strains were detected as shown in the table below

Analytical Reactivity Results for *C. difficile* strains

No.	Strain	Concentration (cfu/mL)	Toxinotype	C.*difficile* Result #Detected / #Total
1	ATCC 17857 (870) A+B+	1.12×10^3	0	3/3
2	ATCC 43594 (W1194) A+B+	1.12×10^3	0	3/3
3	ATCC 43596 (545) A+B+	1.12×10^3	0	3/3
4	ATCC 43597 A+B+	1.12×10^3		3/3
5	ATCC 43598 (1470) A-B+	1.12×10^3	VIII	3/3
6	ATCC 43599 (2022) A+B+	1.12×10^3	0	3/3
7	ATCC 43600 (2149) A+B+	1.12×10^3	0	3/3
8	ATCC 51695 (BDMS 18 AN) A+B+	1.12×10^3	0	3/3
9	ATCC 700792 (14797-2) A+B+	1.12×10^3	0	3/3
10	ATCC 9689 (90556-M6S) A+B+	1.12×10^3	0	3/3
11	ATCC BAA-1382 (630) A+B+	1.12×10^3	0	3/3
12	ATCC BAA-1805 A+B+	1.12×10^3	III	3/3
13	BAA-1814 A+B+	1.12×10^3	XXII	3/3
14	BAA-1870 A+B+	1.12×10^3	III	3/3
15	BAA-1871 A+B+	1.12×10^3	0	3/3
16	BAA-1872 A+B+	1.12×10^3	0	3/3
17	BAA-1873 A+B+	1.12×10^3	0	3/3
18	BAA-1874 A+B+	1.12×10^3	0	3/3
19	BAA-1875 A+B+	1.12×10^3	V	3/3
20	CCUG 8864 A-B+	1.12×10^3	X	3/3

f. Assay cut-off:

Initial reportable range (assay cut-off) was determined by an analysis of Limit of Detection (LoD) Studies and Method Comparison studies during the feasibility phase where 45 cycles of amplification were run. The cycling time was later reduced to 40 cycles as testing demonstrated that samples at LoD were approximately at 38 cycles. Reportable range (assay cut-off) was determined by analysis of a Limit of Detection study and the Method Comparison data generated during assay verification testing. Forty cycles of amplification were performed during assay development to allow for the confirmation of the assay cut off. The Limit of Detection for C difficile was defined as the lowest concentration with ≥95% detection for at least 24 replicates as determined by probit analysis. Analysis of the Limit of Detection study shows that the range of Ct values for the *C. difficile* positive samples at or just above the Limit of Detection was <39. The same LoD sample returned an average Ct value of 36.8. Review of the Method Comparison data showed that 95% of the positive samples have a Ct value <38.7. Samples which were positive for *C. difficile* had Ct values in the range of 23.3 to 39.7. Based on the available data, the assay cut-off was set at Ct = 40.

2. Comparison studies:

a. Method comparison with predicate device:
Clinical specimens were tested using the Simplexa™ *C. difficile* Universal Direct assay and two different FDA cleared assays. A total of 402 samples were assayed using one FDA cleared molecular assay, and 305 samples were assayed using another FDA cleared molecular assay. The testing was performed at two different clinical sites. These two FDA cleared molecular assays and the Simplexa™ C. difficile were compared to direct and enriched toxigenic cultures.

In comparison to direct toxigenic culture, the sensitivity and specificity of the Simplexa™ C. difficile Universal Direct Assay were 90.1% (95% CI:83.8-94.1%) and 93% (95% CI:91-94.5%), respectively. The sensitivities and specificities of the two FDA cleared molecular tests were 86.1% (95% CI:76.3-92.3%) and 94.8% (95% CI:91.9-96.8%) for the first molecular assay and 81.8% (95% CI:65.6-91.4%) and 93% (95% CI:89.3-95.5%), for the second assay.

In comparison to enriched toxigenic culture, the sensitivity and specificity of the Simplexa™ C. difficile Universal Direct Assay were 79.6% (95% CI:73.1-84.8%) and 95.8% (95% CI:94.2-97%), respectively. The sensitivities and specificities of the two FDA cleared molecular tests were 78.7% (95% CI:69.0-85.9%) and 97.1% (95% CI:94.6-98.5%) for the first molecular assay and 69.6% (95% CI:56.7-80.1%) and 97.2% (95% CI:94.3-98.6%), for the second assay. These results met the acceptance criteria and are acceptable.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

A total of 970 prospectively collected stool specimens were obtained from patients with signs and symptoms of *C. difficile* infection from varied geographic locations. Demographic information, including age, gender was noted. Testing was performed at three external testing sites located on the East Coast. Tables below show results comparing the device to direct toxigenic culture method and to enriched toxigenic culture method.

	Reference Method: (Direct Culture + Toxin Assay)		
Simplexa™ C. *difficile* Universal Direct Kit	**Detected**	**Not Detected**	**Total**
Detected	118	59	177
Not Detected	13	779	792
Total	131	838	969
Sensitivity	90.1%(118/131) 95% CI:83.8-94.1%		
Specificity	93.0%(779/838) 95% CI:91.0-94.5%		

	Reference Method: (Enriched Culture + Toxin Assay)		
Simplexa™ C. *difficile* Universal Direct Kit	**Detected**	**Not Detected**	**Total**
Detected	144	33	177
Not Detected	37	755	792
Total	181	788	969
Sensitivity	79.6%(144/181) 95% CI:73.1-84.8%		
Specificity	95.8%(755/788) 95% CI:94.2-97.0%		

Note: One sample was inadvertently missed from being cultured.

The Simplexa *C. difficile* Universal direct Assay showed a sensitivity of 90.1% (83.8 – 94.1 % CI) and a specificity of 93% (91 – 94.5 % CI) when compared to direct toxigenic culture method.

*b. Clinical specificity**:***

See 3a. above

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The prevalence of *C. difficile* varies between institutions. The frequency is affected by patient population, type of institution, and epidemiology. In the

SimplexaTM *C. difficile* Universal Direct prospective study, 177 of the 969 or 18.3% of the samples tested were positive. The percent positivity between the sites varied from 14.9 to 21.6.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.